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10/584,338	01/09/2007	Kevin Allen D'Amour	CYTHERA.045NP	3658

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EXAMINER	
SHEN, WU CHENG WINSTON	

ART UNIT	PAPER NUMBER
1632	

NOTIFICATION DATE	DELIVERY MODE
02/12/2009	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

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Office Action Summary	Application No.	Applicant(s)	
	10/584,338	D'AMOUR ET AL.	
	Examiner	Art Unit	
	WU-CHENG Winston SHEN	1632	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *12/01/2008*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *76-87* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *76-87* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *23 June 2006* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *02/02/2007 and 12/21/2007*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

This application 10/584,338 is a 371 of PCT/US04/43696 filed on 12/23/2004 which claims benefit of 60/532,004 filed on 12/23/2003, and claims benefit of 60/586,566 filed on 07/09/2004 and claims benefit of 60/587,942 filed on 07/14/2004.

Election/Restriction

It is noted that in the Restriction requirement mailed on 10/29/2008, the following groups were listed: Group I, claims 63-65 and 67-72; Group II: claim 73; and Group III: claims 76-87. The Examiner notes that the restriction of Groups I-III was based on the claim set filed on 02/28/2008. However, the Examiner acknowledges that Applicant had filed preliminary claim amendments on 05/13/2008 and claims 1-75 had been cancelled. Therefore, the restriction requiring election of an invention from Groups I-III is moot.

With regard to election of species selected from the group consisting of Nodal, activin A, and activin B (claim 79), in the response filed on 12/01/2008, Applicants elected activin A for prosecution on the merits, without traverse, and Applicants indicated claims 76-87 read on activin A. Applicants states that Applicants request rejoinder of the non-elected species upon allowance of a generic claim and Applicants reserve the right to pursue any or all withdrawn and/or cancelled subject matter in one or more continuing applications.

Claims 76-87 are currently under examination.

Claim Rejection - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

1. Claims 76-87 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method of producing human definitive endoderm cells, said method comprising: obtaining a cell population comprising pluripotent human embryonic stem (hES) cells or human embryonic germ (hEG) cells; and providing said cell population with activin A and Wnt3a, providing serum at concentration lower than 0.2 μg/ml initially to said cell population, thereby generating in said cell population human definitive endoderm cells expressing at least SOX 17 and HNF3β, **does not** reasonably provide enablement of the said methods for (1) producing any mammalian species definitive endoderm cells other than human definitive endoderm cells, (2) obtaining a cell population comprising induced pluripotent stem (iPS) for producing human definitive endoderm cells, (3) providing said cell population with any TGFβ superfamily growth factor in combination with any Wnt-pathway activator other than the TGFβ superfamily growth factor activin A in combination with the Wnt-pathway activator Wnt3a, or (4) providing serum at any concentration for initial culture of pluripotent human cells other than providing serum at concentration lower than 0.2 μg/ml initially to said cell population. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

Enablement is considered in view of the Wands factors (MPEP 2164.01(a)). The court in Wands states: "Enablement is not precluded by the necessity for some experimentation such as routine screening. However, experimentation needed to practice the invention must not be undue experimentation. The key word is 'undue,' not 'experimentation.' " (*Wands*, 8 USPQ2d 1404). Clearly, enablement of a claimed invention cannot be predicated on the basis of quantity of experimentation required to make or use the invention. "Whether undue experimentation is needed is not a single, simple factual determination, but rather is a conclusion reached by weighing many factual considerations." (*Wands*, 8 USPQ2d 1404). The factors to be considered in determining whether undue experimentation is required include: (1) the quantity of experimentation necessary, (2) the amount or direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claims. While all of these factors are considered, a sufficient amount for a *prima facie* case is discussed below.

The nature of the invention is directed to a method for producing human definitive endoderm by directed *in vitro* differentiation of cultured human embryonic stem (hES) cells in the presence of a TGFβ superfamily growth factor and a Wnt-pathway activator.

The breadth of the claims a method for producing a method of producing any definitive endoderm cells, said method comprising: obtaining a cell population comprising any pluripotent human cells; and providing said cell population with any member of TGFβ superfamily growth factor and any member of the Wnt-pathway activators, providing serum at any concentration initially to said cell population.

The specification discloses that Human ES and EG cells (hESCs) offer unique opportunities for investigating early stages of human development as well as for therapeutic

intervention in several disease states, such as diabetes mellitus and Parkinson's disease (See paragraph [0004] of instant application).

With regard to growth factors required for differentiation of hES cells, the specification discloses that in some embodiments of the present invention, one or more growth factors are used in the differentiation process from pluripotent cell to definitive endoderm cell. The one or more growth factors used in the differentiation process can include growth factors from the TGFβ superfamily. In such embodiments, the one or more growth factors comprise the Nodal/Activin and/or the BMP subgroups of the TGFβ superfamily of growth factors. In some embodiments, the one or more growth factors are selected from the group consisting of Nodal, Activin A, Activin B, BMP4, Wnt3a or combinations of any of these growth factors (See paragraph [0010], [0215], and Example 6 of instant application).

With regard to markers of definitive endoderm cells, the specification discloses that in some embodiments, one or more markers selected from **SOX17**, CXCR4, MIXL1, GATA4, **HNF3β**, GSC, FGF17, VWF, CALCR, FOXQ1, CMKOR1 and CRIP1 are expressed in definitive endoderm cells. In other embodiments, one or more markers selected from OCT4, alpha-fetoprotein (AFP), Thrombomodulin (TM), SPARC and SOX7 are not significantly expressed in definitive endoderm cells (See paragraph [0008], Table 1, and Examples 6-11 of instant application).

The specification does not provide enabling support for **(1)** producing any mammalian species definitive endoderm cells other than human definitive endoderm cells, **(2)** obtaining a cell population comprising induced pluripotent stem (iPS) for producing human definitive endoderm cells, **(3)** providing said cell population with any TGFβ superfamily growth factor in

combination with any Wnt-pathway activator other than the TGFβ superfamily growth factor activin A in combination with the Wnt-pathway activator Wnt3a, or **(4)** providing serum at any concentration for initial culture of pluripotent human cells other than providing serum at concentration lower than 0.2 μg/ml initially to said cell population.

(1) It is noted that the preamble of claim 76 recites "A method of producing definitive endoderm" whereas the active step of claim 76 recites "obtaining a cell population comprising pluripotent human cells". Since the specification and the status of art doe not support the embodiment that the recited "pluripotent human cells" can give rise to any definitive endoderm cells of mammalian species other than human (i.e. trans species differentiation), the claimed method is not enabled for producing any mammalian species definitive endoderm cells other than human definitive endoderm cells.

(2) Pertaining to the claimed pluripotent human cell population of claimed method, it is noted that the pluripotent mammalian cell recited in claim 1 reads on induced pluripotent stem cells (iPS), which have not been demonstrated to behave in an identical way as pluripotent embryonic stem cells. Furthermore, the Examiner notes that the specification does not contemplate on using induced pluripotent stem cells (iPS) for the claimed method because the method for generation of induced pluripotent stem cells (iPS) was not substantiated until 2006, which is after the claimed priority date of instant application. In this regard, For instance, **Takahashi et al.** demonstrates the generation of iPS cells from adult human dermal fibroblasts with the expression of the same four factors: Oct3/4, Sox2, Klf4, and c-Myc. Takahashi et al. teaches that human iPS cells were similar to human embryonic stem (ES) cells in morphology, proliferation, surface antigens, gene expression, epigenetic status of pluripotent cell-specific

genes, and telomerase activity, and furthermore, these cells could differentiate into cell types of the three germ layers *in vitro* and in teratomas (See summary, Takahashi et al., Induction of pluripotent stem cells from adult human fibroblasts by defined factors, *Cell* 131(5):861-72, 2007).

(3) With regard to growth factors required for differentiation of hES cells to human definitive endoderm cells, it is worth noting that members of TGFβ superfamily are involved in regulation of various signaling pathways. **Valdimarsdottir et al.** provides the following statements regarding the status of art pertaining to the unpredictable nature of *in vitro* differentiation of hES cells and the role of TGFβ superfamily signaling during differentiation: It is clear that as long as hES cell lines are not clonally (re-) derived and culture conditions differ substantially between laboratories with a resulting degree of selection, it will be difficult to make broad generalizations on the molecular basis of self-renewal and differentiation control. In fact, different lines may be suitable for different purposes. Nevertheless, as the literature grows, some universal principles are emerging that are common to mouse and human ES cells, suggesting that transcriptional control may be conserved although the upstream pathways by which it is regulated may show species specificity. This emphasizes the importance of studying hES cell and mES cells in parallel whenever possible. In addition to their presumed importance in self-renewal, the TGFβ family members have been shown to have multiple regulatory roles in ES cell differentiation. TGFβ superfamily signaling can lead to very different effects, ranging from maintenance of self-renewal to specific differentiation steps. The decision that a cell makes when subjected to a given cytokine might therefore depend on the particular state of the cell upon receiving the signal and on the ligand concentration (See Conclusion, left column, page 784,

Valdimarsdottir et al., Functions of the TGFβ superfamily in human embryonic stem cells. *APMIS*, 113(11-12):773-89, 2005). With regard to activation of Wnt pathway for differentiation of hES cells, **Ciani et al.** teaches that Wnt signaling has a key role in early embryonic patterning through the regulation of cell fate decisions, tissue polarity and cell movements, and that there are three main branches of the Wnt signaling pathway that regulate distinct sets of gene expression (See abstract and Figure 1, Ciani et al., WNTs in the vertebrate nervous system: from patterning to neuronal connectivity, *Nat Rev Neurosci.* 6(5):351-62, 2005). Therefore, in the absence of indication of specific recitation requiring combination of activin A and Wnt3a to initiate hES cells differentiation in the claimed methods, one of skill in the art would have to perform undue experimentation to make and use the claimed invention commensurate in scope with the claims 76-87. This is of particular importance in light of the unpredictability in the art regarding *in vitro* differentiation of hES cells and the fine tuned roles of TGFβ superfamily signaling taught by Valdimarsdottir et al. and the presence of multiple and distinct Wnt signaling pathways taught by Ciani et al.

(4) Pertaining to specific requirement for *in vitro* differentiation of hES cells, the state of the art indicates that the presence of insulin in the medium suppresses definitive endoderm differentiation from human embryonic stem cells. For instance **McLean et al.** reported that contacting human embryonic stem cell with insulin in a concentration as little as 0.2 μg/ml during the differentiation process is detrimental to the production of definitive endoderm (See Fig. 6, Mclean et al., Activin A efficiently specifies definitive endoderm from human embryonic stem cells only when phosphatidylinositol 3-kinase signaling is suppressed, *Stem Cells*, 25(1):29-38, 2007).

As issues pertaining to *in vitro* differentiation of hES cells discussed above, in view of the state of the art, the unpredictability in the art, and the lack of specific guidance and working examples in the specification, one of skill in the art would have to perform undue experimentation to make and use the claimed invention commensurate in scope with the claims 76-87.

Claim Rejection - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless -

(e) the invention was described in (1) an application for patent, published under section 122(b), by another filed in the United States before the invention by the applicant for patent or (2) a patent granted on an application for patent by another filed in the United States before the invention by the applicant for patent, except that an international application filed under the treaty defined in section 351(a) shall have the effects for purposes of this subsection of an application filed in the United States only if the international application designated the United States and was published under Article 21(2) of such treaty in the English language.

2. Claims 76, 77, 79, 80, and 82-87 are rejected under 35 U.S.C. 102(e) as being anticipated by **Fisk et al.** (U.S. Patent 7,326,572, issued 02/05/2008) as evidenced by **Kuo et al.** (Kuo et al., Roles of histone acetyltransferases and deacetylases in gene regulation *Bioessays*, 20(8):615-26, 1998).

Claim 76 is directed to a method of producing definitive endoderm cells, said method comprising: obtaining a cell population comprising pluripotent human cells; and providing said cell population with a TGFβ superfamily growth factor and a Wnt- pathway activator, thereby generating in said cell population definitive endoderm cells expressing at least SOX 17 and HNF3β.

With regard to the limitations of claims 76, 79, 80, 82, 83, 85, and 86 of instant application, Fisk et al. teaches endoderm derived from human embryonic stem cells (See title, Fisk et al.). Fisk et al teaches a method for generating endoderm cells from human embryonic stem (hES) cells or human embryonic germ (hEG) cells, comprising culturing the hES cells or hEG cells in a medium comprising a sufficient amount of Activin A (See Table 1, Fisk et al., which reads on claims 79, 80, 82 and 83 of instant application) to cause differentiation of said cells into endoderm, thereby generating endoderm cells, wherein the endoderm cells express the markers Sox 17, HNF3β and HNF4α (claim 1 of Fisk et al., which reads on definitive endoderm cells expressing at least SOX 17 and HNF3β recited in claim 1 of instant application), wherein the medium further comprises sodium butyrate (claim 2 of Fisk et al., which reads on a Wnt-pathway activator recited in claim 1 of instant application), wherein the cells are human embryonic stem cells (claim 3 of Fisk et al., which reads on pluripotent human cells recited in claim 1 of instant application, and hES cells recited in claims 85 and 86 of instant application), wherein the cells are grown to confluence under feeder-free conditions before the hPS cells are cultured with Activin A (claim 4 of Fisk et al., which reads on "a TGFβ superfamily growth factor" recited in claim 76 of instant application). Fisk discloses that Sox 17 is a marker for identification of definitive endoderm cells and HNF3β is a marker for identification of endoderm cells (See Table 2 of Fisk et al.).

It is noted that Fisk et al. discloses n-butyrate (for instance, sodium butyrate) is a histone deacetylase (HDAC) inhibitor (See Table 1, Fisk et al.). Inhibition of HDAC leading to high acetylation level of histone during chromatin remodeling is known to activate transcription of genes in mammalian genome. Accordingly, n-butyrate is a non-signal-specific Wnt-pathway

activator. The connection between high histone acetylation level and transcription activation is evidenced by **Kuo et al.** (See abstract and Figure 2, Kuo et al., Roles of histone acetyltransferases and deacetylases in gene regulation *Bioessays*, 20(8):615-26, 1998).

With regard to removal of TGFβ superfamily growth factor from said cell population (claim 77 of instant application), Fisk et al. teaches step-wise differentiation steps of hES cells in which activin A is added in the initial media (See column 10-11, and Table 1) to maintain the characteristics of pluripotent hES cells, and at later stages of differentiation the media no longer contains activin A (See for instance, Table 3, Fisk et al.).

With regard to the limitation "providing serum to said cell population in increasing concentration" (claim 84 of instant application), the broad and reasonable interpretation of this limitation reads on switching the old medium from one stage of differentiation to a new media of the next stage of differentiation, for instance Step II to Step III listed in Table 3 of Fisk et al., because fresh media would contain more serum compared to the media that have cells grown in for several days.

With regard the source of hES cells (claim 87 of instant application), Fisk et al. teaches that inner cell mass of embryo is one source of hES cells (See lines 13-29, column 8, Fisk et al.)

Thus, Fisk et al. (U.S. Patent 7,326,572, issued 02/05/2008) as evidenced by Kuo et al. (1998) clearly anticipates claims 76, 77, 79, 80, 82-87 of the instant application.

3. Additional references listed below are related to the claimed inventions.

(1). Fisk et al. US 7,033,831, issued on 04/25/2006

(2). Keller et al., US patent application publication 2006/0003446, publication date 01/05/2006.

(3) Prosecutions of Application 11/021,618 (US 2005/0158853) and Application 12/167,227 (US 2008/0267926).

Conclusion

4. No claim is allowed.

Applicant is reminded that upon the cancellation of claims to a non-elected invention, the inventorship must be amended in compliance with 37 CFR 1.48(b) if one or more of the currently named inventors is no longer an inventor of at least one claim remaining in the application. Any amendment of inventorship must be accompanied by a request under 37 CFR 1.48(b) and by the fee required under 37 CFR 1.17(i).

Any inquiry concerning this communication from the examiner should be directed to Wu-Cheng Winston Shen whose telephone number is (571) 272-3157 and Fax number is 571-273-3157. The examiner can normally be reached on Monday through Friday from 8:00 AM to 4:30 PM. If attempts to reach the examiner by telephone are unsuccessful, the supervisory patent examiner, Peter Paras, Jr. can be reached on (571) 272-4517. The fax number for TC 1600 is (571) 273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private

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/Wu-Cheng Winston Shen/
Patent Examiner
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